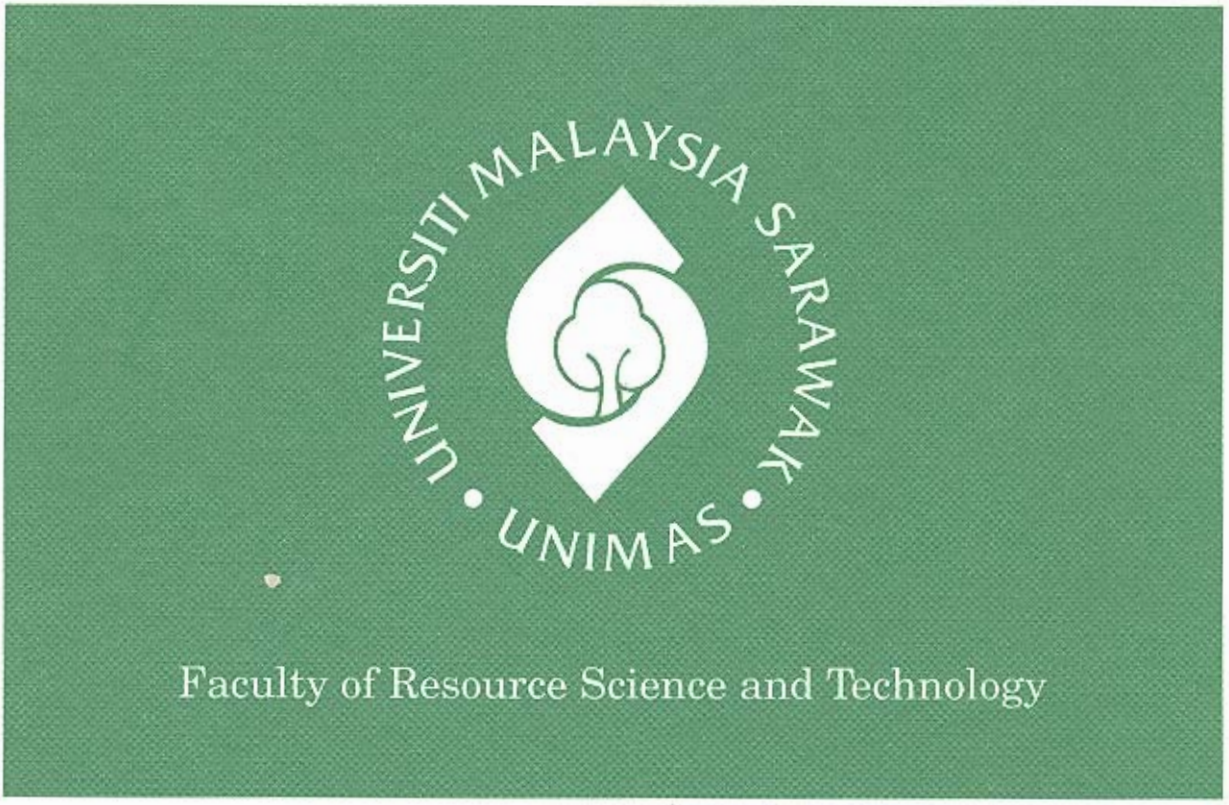

EFFECTS OF POLYPEPTONE IN LACTIC ACID FERMENTATION UTILIZING *LACTOCOCCUS LACTIS* IO-1

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Bachelor of Science with Honours
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Effects of Polypeptone in Lactic Acid Fermentation Utilizing *Lactococcus lactis* IO-1

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ABSTRACT

The effects of four different concentrations of polypeptone were studied in the lactic acid production from fermentation of hydrolyzed sago starch by *Lactococcus lactis* IO-1. The four polypeptone concentrations were 0 g/l, 3 g/l, 5 g/l and 8 g/l. This study was conducted to discover the optimal concentration of polypeptone in lactic acid fermentation which is able to produce the maximum amount of lactic acid. This is also to reduce the cost for the process. From this research, batch fermentations using 3 g/l polypeptone and 5 g/l polypeptone shows only minimal differences in the amount of lactic acid and biomass produced at 55.36 g/l, 0.05 g/g and 54.06 g/l, 0.04 g/g respectively. Without the presence of polypeptone, the glucose residuals are high (22.41 g/l) with the lowest glucose consumption efficiency at 61.14 %. 3 g/l polypeptone was found to produce higher amount of lactate yield, 0.93 g/g compared to 0.56 g/g lactate from 0 g/l polypeptone (control), 0.91 g/g lactate from 5 g/l polypeptone and 0.90 g/g lactate from 8 g/l polypeptone.

Key words: Batch fermentation, *Lactococcus lactis*IO-1, Polypeptone, Lactic acid production.

ABSTRAK

Kesan empat kepekatan polipepton yang berbeza dikaji dalam penghasilan asid laktik melalui proses fermentasi kanji terhidrolisis oleh Lactococcus lactis IO-1. Empat kepekatan polypepton ini ialah 0 g/l, 3 g/l, 5 g/l and 8 g/l. Kajian ini dijalankan untuk mengenalpasti kepekatan polipepton yang optimum dalam fermentasi asid laktik yang boleh menghasilkan amaun asid laktik yang maksimum dan untuk mengurangkan kos dalam proses. Melalui kajian ini, fermentasi kelompok yang menggunakan 3 g/l polipepton dan 5 g/l polipepton menunjukkan hanya sedikit perbezaan dalam amaun asid laktik dan biomas yang dihasilkan iaitu masing-masing pada 55.36 g/l, 0.05 g/g dan 54.06 g/l, 0.04 g/g . Tanpa penggunaan polipepton, baki glukosa adalah tinggi (22.41 g/l) dengan efisiensi pengumpulan glukosa terendah iaitu pada 61.14%. 3 g/l polipepton didapati menghasilkan jumlah asid laktik yang tertinggi iaitu 0.93 g/g berbanding 0.56 g/g asid laktik dari 0 g/l polipepton (kawalan), 0.91 g/g asid laktik dari 5 g/l polypeptone dan 0.90 g/g asid laktik dari 8 g/l polipepton.

Kata kunci: Fermentasi kelompok, Lactococcus lactisIO-1, Polipepton, Penghasilan asid laktik

CHAPTER 1

INTRODUCTION

Fermentation is an anaerobic catabolism of metabolites for energy production (Horton *et al*, 2002). It involves chemical reaction in which sugars are broken down into smaller molecules that can be used in living systems. According to Thieman and Palladino (2004), there are two most common types of fermentation, lactic acid fermentation and alcohol (ethanol) fermentation. Anaerobic bacteria that undergo lactic acid fermentation produce lactic acid as a waste product, while alcohol-fermenting bacteria generate alcohol and carbon dioxide as waste products.

However, lactic acid fermentation has become the most important commercial fermentation processes nowadays. One of the reasons for this statement is that the lactic acid production by the fermentation of homofermentative lactic acid bacteria does not released carbon dioxide (Ishizaki *et al*, 1993) and is therefore environmental friendly. Thus, an increased level of carbon dioxide in atmosphere which causing a green house effect will not be an issue anymore. As mentioned by Tortora *et al* (2004), lactic acid fermentation can produce yoghurt from milk, sauerkraut from fresh cabbage, and pickles from cucumbers. Lactic acid has turned out to be the most significant organic acids which have attracted vast attention due to its high potential to be used in the manufacture of biodegradable plastics (Hipolito, 2001).

In this project, the objective is to study the effect of polypeptone and to reveal the optimum polypeptone concentration in lactic acid fermentation which is able to produce the maximum amount of lactic acid. As reported by Peterson and Pigford (1984), polypeptone is essential as the nitrogen source for the growth of *Lactococcus lactis* IO-1. It allows *Lactococcus lactis* IO-1 to maintain a stable level of growth for some time, indicating that it consists of a larger quantity of the component(s) used by *Lactococcus lactis* IO-1 for development (Hipolito, 2001). However, it was expensive complex nitrogen source practically the same as yeast extract (Pyung *et al*, 2000) and it cost RM600 per kg in the market (Bujang *pers. comm.*). Therefore, the usage of the ideal concentration of polypeptone that can produce the maximum amount of lactic acid should be revealed.

As added by Hipolito (2001), it could be possible to eliminate or at least decrease the concentration of polypeptone to levels of 5 g/l or lower. Laboratory scale fermentation nowadays is using the 5 g/l polypeptone as the standard in initiating the fermentation process. Meantime, elimination of polypeptone also does not ensure whether the production of lactic acid can still be maximized. Therefore, lactic acid fermentation that were using polypeptone and excluding polypeptone will also be analyzed for this purpose. Besides that, the accomplishment of this study can avoid the wastes amount of polypeptone used due to the excess of the polypeptone left during the fermentation. For that reason, the effect of different concentrations of polypeptone will be studied as well. This task will be carried out through batch fermentation process utilizing *Lactococcus lactis* IO-1 and the hydrolyzed sago starch (HSS) will functions as substrate. Furthermore, the well-matched polypeptone

concentration that has been determined will provide the best combination for cost-effective large-scale lactic acid production. It can also offer more benefits in lactic acid fermentation study as well as reducing the cost of the fermentation process.

CHAPTER 2

LITERATURE REVIEW

2.1 Sago

According to Cargill (2004), sago starch which is sourced from the pith of the sago palm was obtained from the genera *Metroxylon sagu* Rottboel and *Metroxylon rumphii* Martins and in minor quantities from the genus *Cycas Linnaeus*. This plant which belong to the Family Palmae and locally known as 'mulong' tree is the only commodity that can grow under the harsh swampy environment which occupies 75% of Sarawak with only minimum care (Bujang and Ahmad, 1999). In 2002, Ishizaki and co-workers reported that sago palm is very efficient in photosynthesizing biomass from carbon dioxide, potentially producing 15-20 t of starch per ha per year. Besides, it is the highest productivity so far recorded when compared to cereals such as rice, corn, wheat and tapioca (Ishizaki, 1997). As added by Bujang (2004), fermenting glucose that is obtained from the bioconversion of sago starch to lactic acid (US$30-50/litre) will certainly add further value to the end product. The bioconversion is a sensible alternative since glucose (US$0.50/kg) fetch a higher price to sago starch (US$0.20/kg). Moreover as a food, sago starch can be used for industries such as bio-fuels, petroleum replacement on plastic, textile, or biodegradable plastic (Ishizaki, 1997) as well as in the production of paper glue without toxin (Bujang and Ahmad, 2000).

2.2 Lactic acid

Lactic acid (2-hydroxypropanoic acid, 2-hydroxypropionic) was first discovered by Scheele in 1780 (Datta *et al*, 1995). As reported by Hipolito (2001), it is the most widely organic hydroxycarboxylic acid easily found widespread in the nature. It plays an important role in chemical and pharmaceutical industries as well as broadly used in food, leather (Vickroy, 1985), cosmetic production, plastics, solvents and textile dyeing, and to aid in preservation (Bujang *et al*, 2001). Petroleum that been used to make plastic can be replaced by lactic acid has been proved achievable by Ishizaki and co-workers (2002) when they discovered that there has been an increased interest in lactic acid production because it could be used as a raw material for the production of poly lactic acid (PLA). PLA is a polymer used as specialty medical and environmental-friendly biodegradable plastics which can be substitution for synthetic plastics derived from petroleum feedstock (Datta *et al*, 1995).

2.3 *Lactococcus lactis* IO-1

Lactic acid bacteria (LAB) are common microbes and are closely related to almost all fields of human life (Ishizaki *et al*. 1990b). These bacteria in the genus *Lactococcus* are able to grow in both anaerobic and microaerophilic conditions. According to Davidson *et al* (1991), lactococci plays an important part in the fermentation industries in producing dairy product such as cheese and yogurt. LAB that will be utilized in this study is *Lactococcus lactis* IO-1

by reason of its favorable characteristic such as gram-positive ovoid coccus, catalase negative and non-motile strain as well as able to grow in 6.5 % NaCl, at 10-45°C (Ishizaki *et al*, 1990b).

In 1993, Ishizaki *et al* reported that *Lactococcus lactis* IO-1 can convert both glucose and xylose to L-lactate effectively and would be a particularly useful organism for the production of lactic acid from hydrolyzed lignocelluloses. Furthermore, due to its homolactic character, the fermentation would not produce carbon dioxide from the degradation of plant biomass and would thus result in, an albeit small, reduction in the atmosphere concentration of this gas (Ishizaki *et al*, 1993). As explained by Ishizaki *et al* (1990a), *Lactococcus lactis* IO-1 produces L-lactic acid as a major metabolic product from glucose and no other significant lower fatty acids. In addition, because of its higher recovery which is up to 90% as mentioned by Ishizaki *et al* (1990b), the high fermentation rate from glucose to L-lactic acid makes it as the preferred selection for industrial application in L-lactate fermentation.

2.4 Batch Fermentation

In batch fermentation, all the nutrients necessary during one run of cultivation except for molecular oxygen in an aerobic process and ammonia or other chemicals for pH adjustment, are added to the medium before cultivation started, and the broth containing the product is withdrawn only at the end of each batch run (Yamane, 1995). According to Scragg (1991), the batch culture systems represent growth in a closed system or environment using a flask or fermentor containing a suitable growth-supporting medium, operated under the optimum temperature, pH and redox potential. The reactor is filled with a sterile nutrient substrate and inoculated with the microorganism. The culture is allowed to grow until no more of the product is being made.

2.5 Polypeptone

Nitrogen source is a very important factor for lactic acid bacteria to promote the growing and the productivities in the lactic acid fermentation (Hipolito, 2001). Polypeptone functions as one of the nitrogen sources in the growth of *Lactococcus lactis* IO-1 in fermentation medium (Peterson and Pigford, 1984) besides yeast extract acid fermentation. In 1999, Pyung *et al* reported that polypeptone enhanced production of metabolites by anaerobic bacteria. Therefore, the actual concentrations of polypeptone that can effectively produce the maximum amount of lactic acid are desirable. This is because if the polypeptone

concentration does not adequate to the growth of the *Lactococcus lactis* IO-1, it will results in less production of lactic acid. The effects of the polypeptone for the growth of the bacteria as stated by Pyung *et al* (1999) approved that polypeptone has providing the complex nitrogen source which is important for the cultivation of anaerobic bacteria and production of their metabolites. Thus, the successful completion of this study will able to avoid the excess amount of costly polypeptone used during the fermentation process.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sago Starch

Food grade sago starch powder was obtained from the local market.

3.2 Polypeptone

Polypeptone was supplied by BBL (Becton Dickinson & Company, Cockeysville, MD 21030, USA). Different concentrations of polypeptone (0 g/l, 3 g/l, 5 g/l, and 8 g/l) were prepared in the early stage of the experiment.

3.3 *Lactococcus lactis* IO-1

The microorganism utilized was *Lactococcus lactis* IO-1, (JCM) 7638. Upon activation, *Lactococcus lactis* IO-1 was taken out from stock culture (-84°C). The microorganism was transferred to Thioglycolate (TGC) medium without Dextrose (Difco, USA) and was incubated for 18 hours at 37°C for maximum growth rate (Ishizaki *et al*, 1990b). The

microorganism was subcultured at least two times before used as stock cultures or in fermentation process.

3.4 Hydrolysis of Sago Starch

Two types of commercial enzymes used for hydrolysis of sago starch were Termamyl-120L (thermostable amylase from *Bacillus lincheniformis*, 120 KNU/g) and AMG 300 L (glucoamylase from *Aspergillus niger)* supplied by Novo Nordisk. The procedure has been detailed elsewhere (Bujang *et al*, 1999) Refer Appendix 1.

3.5 Culture Media

Basal media for fermentation was consist of yeast extract 5 g/l, four different concentrations of polypeptone (0 g/l, 3 g/l, 5 g/l, and 8 g/l), 5 g/L sodium chloride and glucose 60 g/L (from Hydrolyzed Sago Starch, HSS). The same medium with 10 g/L glucose, 5 g/L yeast extract, 5 g/L polypeptone and 5 g/L sodium chloride was used for inoculum preparation. Seed culture for all fermentation trials was 10% (v/v) inoculum (Ishizaki and Ohta, 1989). It was prepared by added 10% (v/v) of the refreshed cells to the inoculum media prior to incubation at 37°C for 6 hours with an agitation speed of 200 rpm.

3.6 Fermentation Process

Fermentation was performed using 1.2 L fermentor jar equipped with polystat cc2 and Cimarec magnetic stirrer with a 1 L working volume (Refer to Plate 1 and 2). The pH probe was calibrated prior to autoclaving. Fermentation system was conducted for 30 hours with controlled pH at 6, temperature at 37°C and agitation rate at 500 rpm. For sampling, 10 ml broth was extracted manually every six hours starting from 0 hour until 30 hours. Samples were kept at 4°C prior to analysis.

Plate 1: Fermentation setup

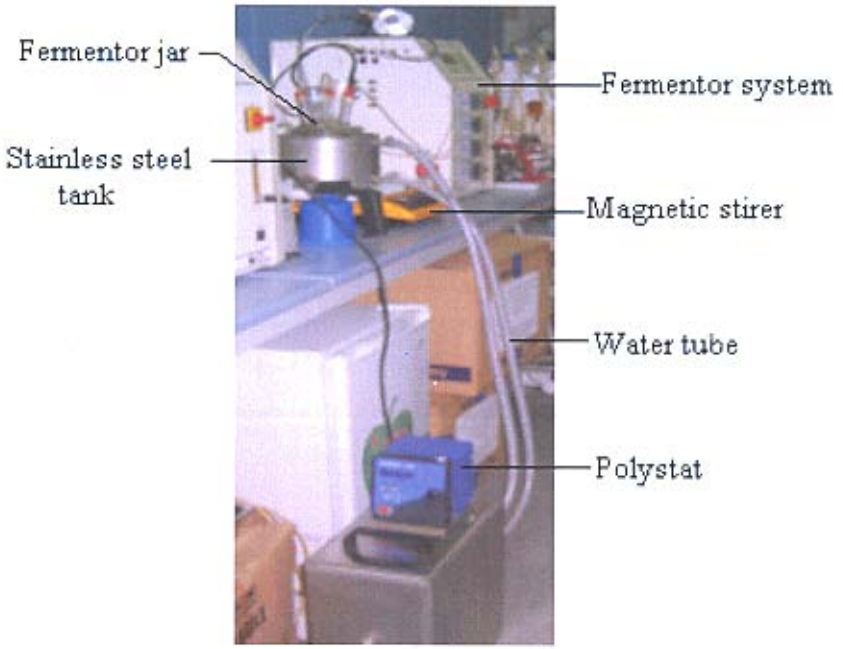

Plate 2: Fermentation setup with polystat

3.7 Analytical Techniques

3.7.1 Dried Cell Weight Determination

Dried cell weight (DCW) method was done to determine the growth of *Lactococcus lactis* IO-1 (Appendix 2).

3.7.2 Reducing Sugar Analysis

Reducing sugar was analyzed using the Dinitrosalicyclic acid (DNS) method (Miller, 1959), (Appendix 2).

3.7.3 Lactic Acid Determination

Lactic acid was analyzed using HPLC (Appendix 2).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Effects of 0 g/l polypeptone concentration (control)

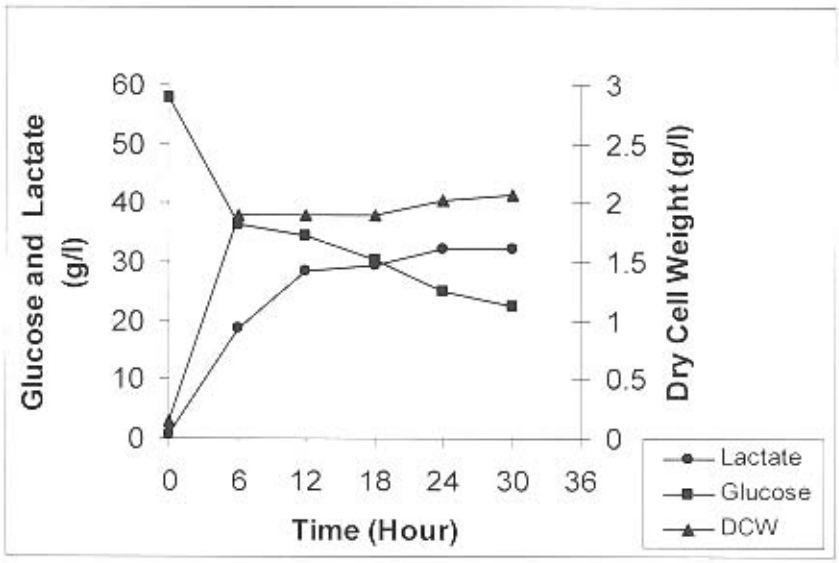

Figure 4.1: Batch fermentation utilizing 0 g/l polypeptone concentration

4.1.2 Effects of 3 g/l polypeptone concentration

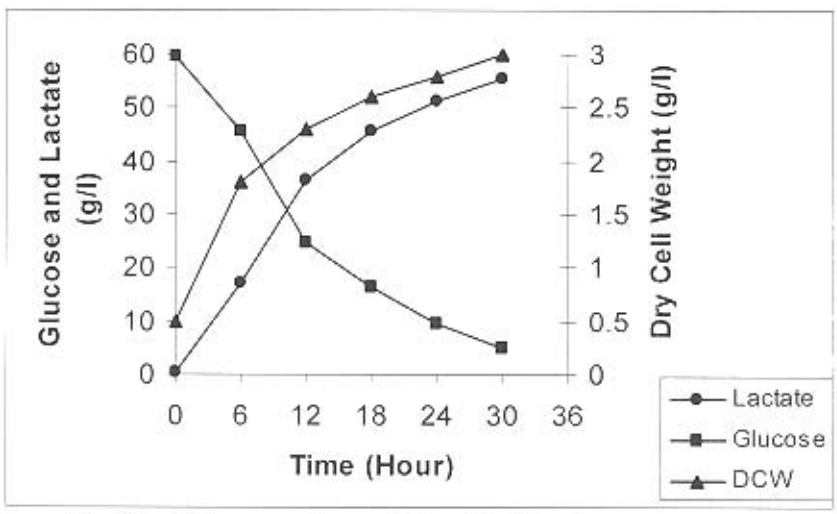

Figure 4.2: Batch fermentation utilizing 3 g/l polypeptone concentration

4.1.3 Effects of 5 g/l polypeptone concentration

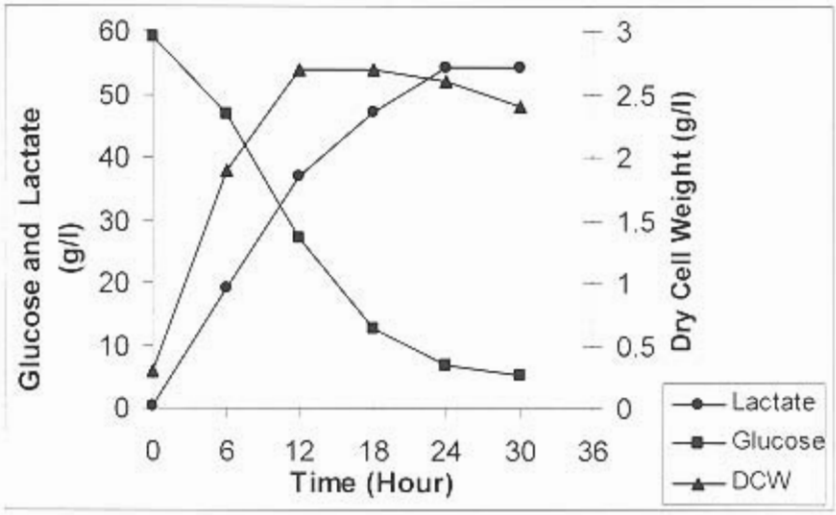

Figure 4.3: Batch fermentation utilizing 5 g/l polypeptone concentration

4.1.4 Effects of 8 g/l polypeptone concentration

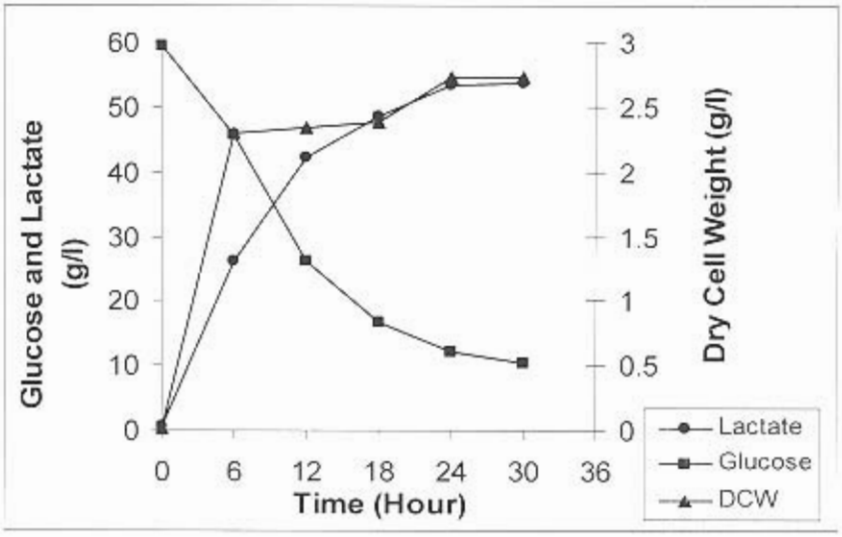

Figure 4.4: Batch fermentation utilizing 8 g/l polypeptone concentration